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13. ABSTRACT (Maximum 200 Words) This report describes the progress achieved during the third year of the project. We proposed to complete Task 3 and to implement Task 4 in the third year of the project. Task 3 focuses on in vivo studies of efficacy of cancer therapy with the use of ultrasound-enhanced delivery of anti-cancer drug 5-FU (low-molecular weight drug) in human MCF-7 breast tumors of nude mice. We started to implement Task 4 which is devoted to in vivo studies of efficacy of cancer therapy with ultrasound-enhanced delivery of macromolecular anti-cancer drug Interleukin-2 (anti-cancer drug with high molecular weight). We partially conducted these studies to implement the proposed tasks. Our data obtained during these chronic experiments indicate that this technique may significantly improve breast cancer therapy. We have found that volume of ultrasound-irradiated tumors decreases when mice are injected with anti-cancer drug in combination with cavitation-mediating agent, while non-irradiated tumors of same mice grow. No improvement in cancer therapy was obtained when no cavitation-mediating agent was used. These results are very encouraging. To complete these studies, we requested a no-cost extension which was granted. The proposed studies will be implemented by the end of the project.				
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INTRODUCTION

Breast cancer is a major health problem in the USA. Despite the recent progress in the development of promising anti-cancer chemo- and biotherapeutic agents, no breakthrough has been achieved in breast tumor therapy and therapy of tumors in other organs. The efficacy of anti-cancer drugs (especially most promising macromolecular agents) is limited due to their poor penetration through tumor capillary walls, interstitium, and cancer cell membranes [1, 2].

The objective of the proposed research is to develop and test a novel drug delivery technique utilizing interaction of ultrasound with exogenous microparticles that can produce cavitation in tumors [3]. The interaction of ultrasound with the microparticles results in cavitation (formation, growth, and collapse of microbubbles) in tumors without damage to normal tissues. The microparticles serve as cavitation nuclei that substantially lower cavitation threshold selectively in tumors [4-8]. The ultrasound-induced cavitation perforates tumor blood vessel walls and cancer cell membranes and induces microconvection in the interstitium that enhances penetration of anti-cancer drugs in the cancer cells.

The focus of this research project is to study ultrasound-enhanced delivery of model and real drugs in human breast tumors and perform breast cancer chemo- and biotherapy by using the ultrasound-enhanced drug delivery. The studies are performed *in vivo* in nude mice bearing human MCF-7 breast tumors.

The third year of the project focuses on the chronic studies of efficacy of cancer therapy with the use of ultrasound-enhanced delivery of anti-cancer drugs with low and high molecular weights (5-FU and Interleukin-2, respectively) by using evaluation of growth of irradiated and control tumors after the treatment.

BODY

Materials and Methods

The studies were performed in athymic nude mice (average weight of 30 g). These studies were approved by the Institutional Animal Care and Use Committee (IACUC) of UTMB.

Suspensions of human breast MCF-7 cancer cells (15×10^6 per site) were injected s.c. in the dorso-scapular area on the left and right sides of each mouse. Experiments were initiated when tumors reached the size of 5 to 8 mm.

One tumor was irradiated by ultrasound for 10 minutes, while the other tumor served as control. Ultrasound contrast agent Optison was used as a cavitation-mediating agent. Optison was injected in the tail vein of nude mice prior to irradiation.

Anti-cancer drug 5-Fluorouracil (5-FU) was used in the studies of efficacy of cancer therapy as an anti-cancer drug with low molecular weight (M.W. = 130). The drug was injected i.p. prior to irradiation at the dose of 90 mg/kg which is the optimal dose for cancer therapy in nude mice. Interleukin-2 (MW = 15,500) is used as a macromolecular anti-cancer drug in our studies. Interleukin-2 is injected at the dose 600,000 IU/kg in these studies.

Tumor volume, V , is calculated by the formula: $V = L \times W^2 / 2$, where L and W is the length and the width of the tumor, respectively. This formula is commonly used for the calculation of tumor volume in cancer therapy studies.

Results

Our histological studies of the breast MCF-7 tumor slices stained with H&E (Fig .1) revealed: viable tumor cells with well-defined nuclei in control (non-irradiated) tumors of mice injected with 5-FU and Optison (Fig. 1, left). Very minor necrosis was produced by 5-FU in non-irradiated tumors in small areas of the tumors of these mice. Dramatic necrosis was produced in large areas in irradiated tumors when ultrasound was used in combination with 5-FU and Optison (Fig.1, right): central and right parts of the picture.

Our chronic studies demonstrated significant improvement of breast cancer therapy when ultrasound was used in combination with Optison and 5-FU injections. Fig. 2 shows substantial decrease of irradiated tumor volume, while there were no changes in the non-irradiated (control) tumor volume of the same mouse. These data indicate that ultrasound-enhanced drug delivery results in more efficient cancer chemotherapy.

In contrast, if no anti-cancer drug was used and only Optison was injected, no difference in tumor growth was noticed in tumors of mice of this group. Fig. 3 demonstrates that the volume of irradiated and non-irradiated tumors increases at the same rate. This is because no anti-cancer drug was injected.

KEY RESEARCH ACCOMPLISHMENTS

1. We are conducting chronic studies on ultrasound-enhanced delivery of real anti-cancer drugs (FU-5, an anti-cancer drug with low molecular weight, widely used for cancer therapy and Interleukin-2, a macromolecular anti-cancer drug) in human breast tumors of nude mice. The obtained data indicate that: (1) combination of 5-FU and Optison injections results in enhanced delivery of anti-cancer drug in ultrasound-irradiated tumors; and (2) efficacy of cancer therapy is low if this technique is not applied.
2. We initiated studies of efficacy of breast cancer therapy with the use of ultrasound-enhanced drug delivery of macromolecular anti-cancer drug Interleukin-2.

The results of these studies suggest that this novel drug delivery technique substantially improves delivery of anti-cancer drugs in the breast tumors and may improve efficacy of the breast cancer chemotherapy.

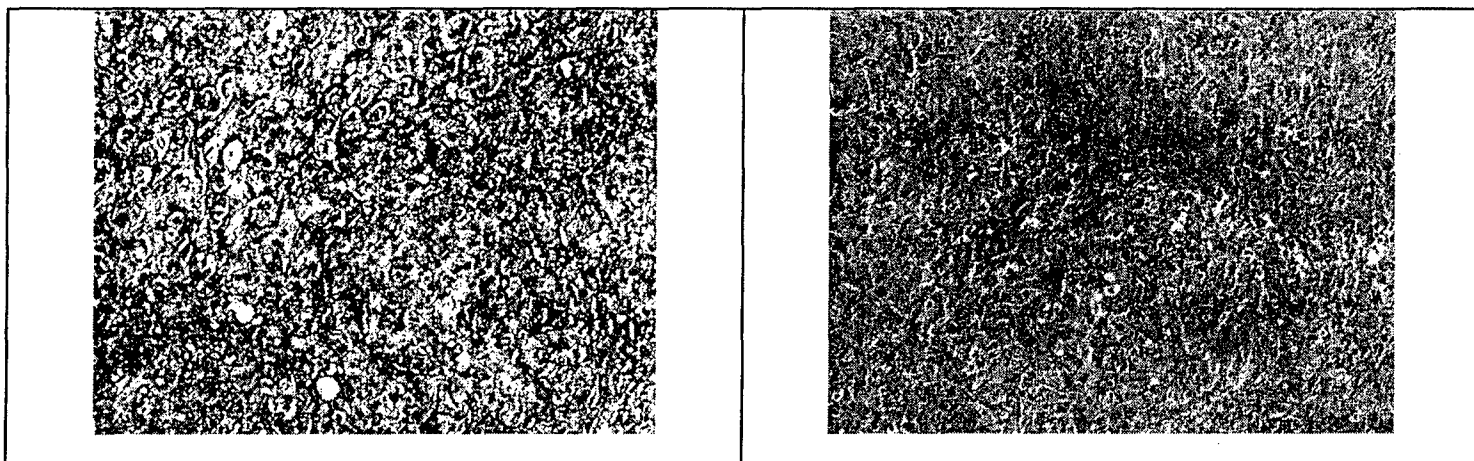


Figure 1. Microscopic histological examination of: (1) control (non-irradiated) MCF-7 breast tumors of nude mouse injected with anti-cancer drug 5-FU and Optison (left) and (b) irradiated breast tumor (right).

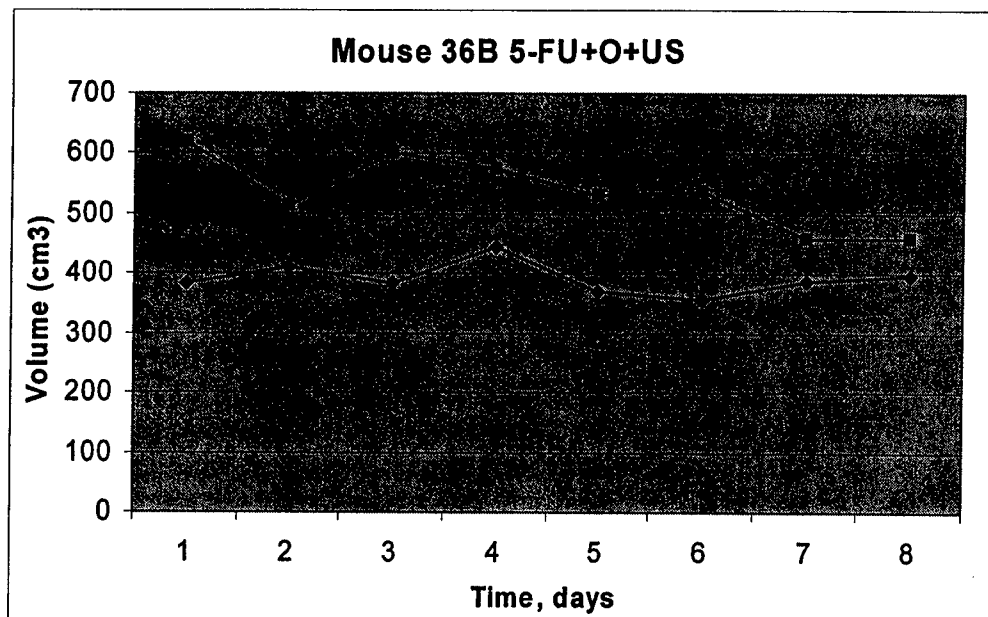


Figure 2. Tumor volume of a mouse injected with anti-cancer drug 5-FU and Optison. Data for irradiated and control tumors are presented by red and blue dots, respectively.

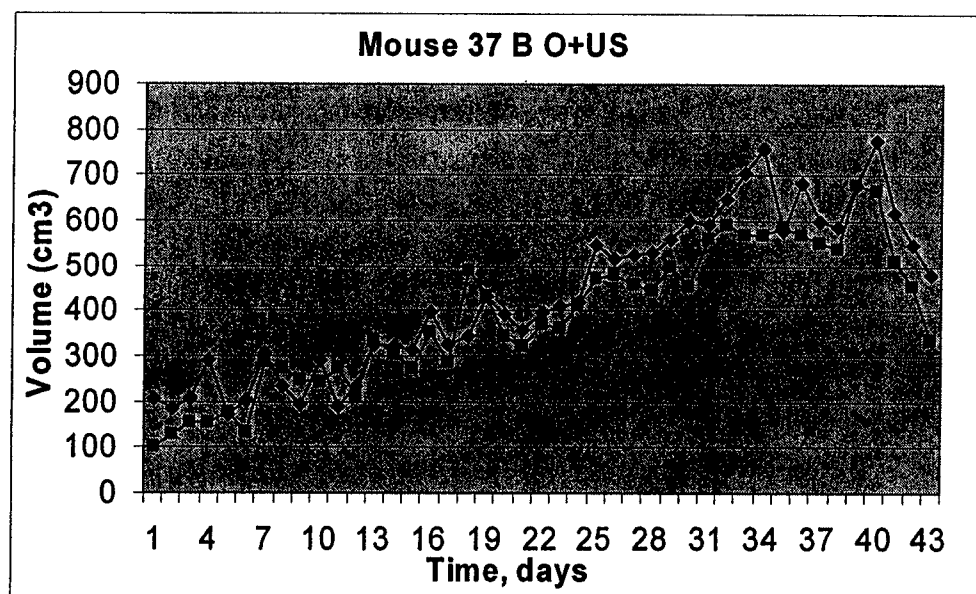


Figure 3. Tumor volume of a mouse injected with Optison only. Data for irradiated and control tumors are presented by red and blue dots, respectively.

REPORTABLE OUTCOMES

During the third year of the project, we had four presentations at two conferences: Annual Conference of Houston Society for Biomedical Engineering and 31st Annual Meeting of Controlled Release Society:

1. Larina I.V., Evers B.M., Ashitkov T.V., Bartels C., Larin K.V., Esenaliev R.O. Delivery of Macromolecular Drugs and Genes in Cancer Cells by Ultrasound-Induced Cavitation. XXI Annual Houston Conference on Biomedical Engineering Research, February-2004.
2. Vassilieva A.A., Evers B.M., Murillo C.A., Esenaliev R.O. Improving the Delivery of Anti-Cancer Drugs with Ultrasound-Induced Cavitation: Animal Studies. XXI Annual Houston Conference on Biomedical Engineering Research, February-2004.
3. Chumakova O.V., Larina I.V., Esenaliev R.O. Ultrasound-Induced Apoptosis in Breast Cancer Cells. XXI Annual Houston Conference on Biomedical Engineering Research, February-2004.
4. Esenaliev R.O. Ivanova Y., Ashitkov T., Evers B.M. Nanoparticles and Ultrasound for Delivery of Macromolecular Drugs in Tumors. 31st Annual Meeting of Controlled Release Society, June-2004.

The results of the studies were published in the Controlled Release Society Conference Proceedings [7]:

1. Esenaliev R.O. Ivanova Y., Ashitkov T., Evers B.M. Nanoparticles and Ultrasound for Delivery of Macromolecular Drugs in Tumors. 31st Annual Meeting of Controlled Release Society, Summary #135, June-2004.

The PDF file of this publication is enclosed.

The results of the studies performed in the third year of the project will be published in two articles that are in preparation for submission to two peer-reviewed journals.

CONCLUSIONS

The results of our studies performed in the third year of the project demonstrated that interaction of ultrasound with nanoparticles results in substantial improvement of breast cancer therapy. Our chronic studies indicated that: combination of 5-FU and Optison injections results in enhanced delivery of anti-cancer drug in ultrasound-irradiated tumors and that efficacy of cancer therapy is low if this technique is not applied. These data suggest that interaction of ultrasound radiation with nanoparticles may substantially improve the efficacy of breast cancer chemotherapy.

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Nanoparticles and Ultrasound for Delivery of Macromolecular Drugs in Tumors

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ABSTRACT SUMMARY

We studied delivery of model macromolecular anti-cancer drugs in mice bearing human colon and breast tumors with cavitation induced by interaction of polymer nanoparticles with ultrasound. Our studies demonstrated enhanced penetration of the drugs from blood into tumor interstitium. The results suggest that this technique may provide efficient cancer chemotherapy.

INTRODUCTION

Efficacy and safety of cancer chemo- and biotherapy are limited, in part, due to poor penetration of macromolecular anti-cancer agents from blood into tumor cells through the physiological barriers: blood vessel wall, interstitial space, and cancer cell membrane [1, 2]. Recently, we proposed to use cavitation (formation, growth, and collapse of microbubbles) to enhance delivery of anti-cancer agents from blood into tumor cells through these physiological barriers [3]. The cavitation can be produced by interaction of tensile pressure of ultrasonic waves with polymer nanoparticles that serve as cavitation nuclei decreasing cavitation threshold [4-6]. High concentration of the nanoparticles can be achieved in tumor capillaries by passive (due to increased leakage of tumor vasculature) or active (by using antibodies directed against tumor vasculature) delivery. The ultrasound-induced cavitation can perforate tumor blood vessel walls and cancer cell membranes and produce microconvection in the interstitium. This may result in enhanced delivery of the anti-cancer drugs in tumor cells.

We quantitatively studied delivery of model macromolecular anti-cancer agents with different molecular weight in human tumors of nude mice by using the ultrasound-induced cavitation.

METHODOLOGY

Suspensions of human colon KM20 or breast MCF-7 cancer cells were injected s.c. in the dorso-scapular area on the left and right sides of athymic nude mice. When tumors reached the size of 5 to 10 mm, one of them was irradiated by ultrasound, while

the other tumor served as control. Polystyrene nanoparticles (10% w/w in water, dia. = 100 nm) were injected in the tail vein of nude mice one day prior to irradiation to allow for extravasation of the particles in tumor blood vessels. Fluorescent rhodamine-dextrans with molecular weights of 10, 70, 2,000 kDa were used to simulate anti-cancer agents: antisense oligonucleotides, antibodies, and genes, respectively. Immunohistochemical staining of thin sections of the irradiated and control tumors was performed to visualize tumor blood vessels. The fluorescence and immunohistochemical staining data were used to calculate the penetration depth of the macromolecules in the interstitium. The penetration depth was measured at 1/e level of fluorescence intensity from the capillary wall.

RESULTS AND DISCUSSION

Analysis of the fluorescence and immunohistochemistry data indicated that rhodamine-dextrans were confined within blood vessels of control (non -irradiated) tumors. Combination of ultrasound with nanoparticle injection resulted in dramatic enhancement of delivery of the macromolecules in the irradiated tumors.

The penetration depth of the rhodamine -dextrans in control tumors decreased with molecular weight: 16, 10, and 3 microns for 10, 70, and 2,000 kDa, respectively. When ultrasound was applied in combination with nanoparticles, the penetration depth increased dramatically: up to 35 microns and was not dependent on the molecular weight.

CONCLUSION

Our studies demonstrated that the ultrasound-induced cavitation substantially enhances delivery of macromolecules in the colon and breast tumor interstitium. This technique may be used for delivery of macromolecular anti-cancer agents in tumor cells of other organs.

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